

Amendments to the Specification

Please delete the two paragraphs beginning on page 4, line 33, and ending on page 5, line 12, and replace them with the following two paragraphs:

FIG. 7 depicts sequences of oligonucleotide primers used in PCRs for the construction of the chCE7 heavy chain gene: CE7VHPCR1.fwd (SEQ ID NO:1), CE7VHPCR2.fwd (SEQ ID NO:2), CE7VHPCR (1+2).rev (SEQ ID NO:3), hGamma1CH1.fwd (SEQ ID NO:4), hGamma1CH1, rev (SEQ ID NO:5), hGamma1CH2.fwd (SEQ ID NO:6), hGamma1CH2.rev (SEQ ID NO:7), hGamma1CH3.fwd (SEQ ID NO:8), hGamma1CH3.rev (SEQ ID NO:9). Forward and reverse primers are identified by the suffixes ".fwd" and ".rev", respectively. Overlaps between different primers, necessary to carry out secondary PCR steps using the product of a primary PCR step as a template, are indicated. Restriction sites introduced, sequences annealing to the CE7 chimeric genomic DNA, and the synthetic leader sequence introduced, are also indicated.

FIG. 8 depicts sequences of oligonucleotide primers used in PCRs for the construction of the chCE7 light chain gene: CE7VLPCR1.fwd (SEQ ID NO: 10), CE7VLPCR2.fwd (SEQ ID NO:11), CE7VLPCR(1+2).rev (SEQ ID NO:12), hKappa.fwd (SEQ ID NO:13), hKappa.rev (SEQ ID NO: 14). Forward and reverse primers are identified by the suffixes ".fwd" and ".rev" respectively. Overlaps between different primers, necessary to carry out secondary PCR steps using as a template the product of a primary PCR step, are indicated. Restriction sites introduced, sequences annealing to the CE7 chimeric genomic DNA, and the leader sequence introduced, are also indicated.

Please delete the paragraph beginning on page 6, line 3, and replace it with the following paragraph:

FIGURE 11 depicts N-linked oligosaccharide biosynthetic pathway leading to bisected complex and bisected hybrid oligosaccharides *via* GnT III-catalyzed reactions. M stands for mannose; Gn N-acetylglucosamine (GlcNAc); G, galactose; Gn^b, bisecting GlcNAc; f, fucose. The oligosaccharide nomenclature consists of enumerating the M, Gn, and G residues attached to the common oligosaccharide and indicating the presence of bisecting GlcNAc by including Gn^b. The oligosaccharide core is itself composed of 2 Gn residues and may or may not include a fucose. The major classes of oligosaccharides are shown inside dotted frames. Man I stands for Golgi mannosidase; ~~TnT~~ GnT, GlcNAc transferase; and GalT, for galactosyltransferase. The mass associated with major, sodium-associated oligosaccharide ion that is observed in MALDI/TOF-MS analysis is shown beside each oligosaccharide. For oligosaccharides which can potentially be core-fucosylated, the masses associated with both fucosylated (+f) and non-fucosylated (-f) forms are shown.

Please replace the paragraph beginning on page 6, line 22, with the following paragraph:

FIGURE 13 depicts the GnT III expression of different cultures of CHO-tet-GnTIII grown at different tetracycline concentrations used to produce distinct C2B8 antibody samples. Cell lysates from each culture grown at 2000 ng/mL (~~Lane C~~) (Lane B), 50 ng/mL (Lane C), and 25 ng/mL (Lane D) tetracycline concentrations were resolved by SDS-PAGE, blotted onto a membrane, and probed with 9E10 (*see supra*) and anti-mouse horseradish peroxidase as primary and secondary antibodies, respectively. Lane A depicts negative control.

Please replace the four paragraphs beginning on page 32, line 26, and ending at page 34, line 15, with the following paragraphs:

Purification Of chCE7 Antibody Samples. Antibody was purified from culture medium by Protein A affinity chromatography on a 1 ml ~~HiTrap~~ Protein A column (HI-TRAP, Pharmacia Biotech, Uppsala, Sweden), using linear pH gradient elution from 20 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, 0.01% ~~Tween~~ 20 polyoxyethylenesorbitan monolaurate (TWEEN 20), 1M urea, pH 7.5 (buffer A) to buffer B (buffer A without sodium phosphate, pH 2.5). Affinity purified chCE7 samples were buffer exchanged to PBS on a 1 ml ~~ResourceS~~ cation exchange column (RESOURCES, Pharmacia Biotech, Uppsala, Sweden). Final purity was judged to be higher than 95% from SDS-PAGE and Coomassie-Blue staining. The concentration of each sample was estimated from the absorbance at 280 nm.

Binding Of antibodies To Neuroblastoma Cells. Binding affinity to human neuroblastoma cells was estimated from displacement of ¹²⁵I-labeled chCE7 by the CHO-produced samples. Amstutz et al, 1993, *supra*.

Oligosaccharide Analysis By MALDLI/TOF-MS. CE7-2000t, -60t, -30t, and -15t samples were treated with *A. urefaciens* sialidase (Oxford Glycosciences, Oxford, United Kingdom), following the manufacturer's instructions, to remove any sialic acid monosaccharide residues. The sialidase digests were then treated with peptide N-glycosidase F (PNGaseF, Oxford Glycosciences, Oxford, United Kingdom), following the manufacturer's instructions, to release the N-linked oligosaccharides. Protein, detergents, and salts were removed by passing the digests through microcolumns containing, from top to bottom, 20 ml of ~~SepPak~~ C18 reverse phase matrix (SEPPAK, Waters, Milford, MA), 20 ml of ~~Dowex AG-50W-X8~~ cation exchange matrix (DOWEX

AG 50W X8, BioRad, Hercules, CA), and 20 ml of AG-4X4 anion exchange matrix (AG 4X4 BioRad, Hercules, Calif.). The microcolumns were made by packing the matrices in a Gel-Loader gel loading pipette tip (GEL LOADER, Eppendorf, Basel, Switzerland) filled with ethanol, followed by an equilibration with water. Kuster *et al.*, 1997, *Anal. Biochem.* 250:82-101. Flow through liquid and a 300 ml-water wash were pooled, filtered, evaporated to dryness at room temperature, and resuspended in 2 ml of deionized water. One microliter was applied to a MALDI-MS sample plate (Perseptive Biosystems, Farmingham, MA) and mixed with 1 ml of a 10 mg/ml dehydrobenzoic acid (DHB, Aldrich, Milwaukee, Wis.) solution in acetonitrile. The samples were air dried and the resulting crystals were dissolved in 0.2 ml of ethanol and allowed to recrystallize by air drying. Harvey, 1993, *Rapid Mass. Spectrom.* 7:614-619. The oligosaccharide samples were then analyzed by matrix-assisted laser desorption ionization/time-of-flight-mass spectrometry (MALDI/TOF-MS) using an Elite-Voyager 400 a mass spectrometer (ELITE VOYAGER 400, Perseptive Biosystems, Farmingham, MA), equipped with a delayed ion extraction MALDI-ion source, in positive ion and reflector modes, with an acceleration voltage of 20 kV. One hundred and twenty eight scans were averaged. Bisected biantennary complex oligosaccharide structures were assigned to five-HexNAc-associated peaks. Non-bisected tri-antennary N-linked oligosaccharides, the alternative five HexNAc-containing isomers, have never been found in the Fc region of IgGs and their syntheses are catalyzed by glycosyltransferases discrete from GnT III.

ADCC Activity Assay. Lysis of IMR-32 human neuroblastoma cells (target) by human lymphocytes (effector), at a target:effector ratio of 1:19, during a 16 h incubation at 37°C. in the presence of different concentrations of chCE7 samples, was measured via retention of a fluorescent dye. Kolber *et al*, 1988, *J. Immunol. Methods* 108: 255-264.

IMR-32 cells were labeled with the fluorescent dye Calcein AM for 20 min (final concentration 3.3 μ M). The labeled cells (80,000 cells/well) were incubated for 1 h with different concentrations of CE7 antibody. Then, monocyte depleted mononuclear cells were added (1,500,000 cells/well) and the cell mixture was incubated for 16 h at 37°C. in a 5% CO₂ atmosphere. The supernatant was discarded and the cells were washed once with HBSS and lysed in ~~Triton X-100 (0.1%)~~ non-ionic detergent, t-octylphenoxypolyethoxyethanol (TRITON X-100 (0.1%)). Retention of the fluorescent dye in IMR-32 cells was measured with a fluorometer (Perkin Elmer, Luminescence Spectrometer LS 50B, (Foster City, Calif.) and specific lysis was calculated relative to a total lysis control, resulting from exposure of the target to a detergent instead of exposure to antibody. The signal in the absence of antibody was set to 0% cytotoxicity. Each antibody concentration was analyzed by triplicate, and the assay was repeated three separate times.